AN IN SILICO APPROACH TOWARDS ACUTE MYELOID LEUKEMIA (WITH FLT3-ITD MUTATION) THERAPY USING HSP 90 AS TARGET

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Certificate

This is to certify that the thesis entitled "**AN IN SILICO APPROACH TOWARDS ACUTE MYELOID LEUKEMIA (WITH FLT3-ITD MUTATION) THERAPY USING HSP 90 AS TARGET."** by **Anurag jha (110BT0025)** submitted to the National Institute of Technology, Rourkela for the Degree of Bachelor of Technology is a record of bonafide research work, carried out by his in the Department of Biotechnology and Medical Engineering under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/ Institute for the award of any Degree or Diploma.

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ABSTRACT

About 20% of cases of acute myeloid leukemia (AML) show an internal tandem duplication of the juxta-position membrane domain of the protein FLT3 which belongs to the family of ligand receptor tyrosine kinase. The disease shows correlation with leukocytosis and poor prognosis as well as.Hsp 90 Inhibitors dissociated FLT3-ITD from the Hsp 90 chaperone complex and hence targeting the Hsp 90 could serve as anti-FLT3ITD therapy, and that the chaperone Hsp 90 is involved closely with the oncogenic activation of FLT3.The inhibitors gave best results at binding site 400 in general. Coumermycin A1 showed the best result with binding energy of -8.1 kcal/mol at the binding site 138 of the Hsp 90.Clorobiocin was yet another compound that showed promising results with energy value of -7.2 kcal/mol at binding site 400.Novobiocin was showed good results and is one of the most important used inhibitor in clinical trials currently in use.

KEY WORDS: Acute myeloid leukemia, FLT3-ITD, Hsp 90, Hsp 90 inhibitors

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CHAPTER 1

INTRODUCTION

1. Introduction:

1.1.1 ACUTE MYELOID LEUKEMIA:

AML (Acute myeloid leukemia) is a category of blood cancer. It generally develops from cells that turn into white blood cells (excluding lymphocytes cells). Sometimes, it can also develop from various types of blood-forming cells. Without treatment, AML can be very fatal. as it is acute medical condition which can spread quickly through the blood and to the to the other parts of the body. It generally affects the Brain and spinal cord, lymph nodes, liver, spleen,testis,etc.The outcome of the disease depends on many factors like age, history of cancer or blood disorder in family lineage and gene mutation.

1.1.2 Risk Factors for Acute Myeloid Leukemia:

Acute myeloid leukemia risk factors include:Smoking, exposure to chemicals, use of chemotherapy, exposure to radiation, blood disorder congenital syndrome, sex(male population is more affected by this disease). Among most of the population, the cause of AML is unknown. It cannot be prevented but we can avoid it by quitting smoking and staying away from high radiation.

1.1.3 Heat shock protein 90 (Hsp 90):

Heat shock protein 90 (Hsp 90) is a molecular chaperone that plays important role in folding, stabilizing, activating and maintaining the conformational integrity of its client proteins through its ATPase activity. Cancer cells employ the use of Hsp 90 chaperone for protection of the the mutated onco-proteins from mis-folding and proteasomal degradation. It is found that Hsp 90 ATPase activity is up regulated nearly 100 fold in cancer cells. And the reason may be the up regulation of its function Hsp 90 is a member of superfamily (includes DNA gyrase, Histidine kinase and DNA mismatch pair) that contains an ATP binding pocket which is different from ATP binding cleft of protein kinases. The conserved structure of the chaperone consists of three domains. These domains are composed of nearly 732 amino acids. It has two isomers α and β, mainly present in cytosol. The N –terminal domain is an amino acid terminal domain that contains a fold known as bergerat fold which is having the ATP and drug binding site. The middle domain has a co-chaperone interacting motifs that provide sites for docking of the client proteins and its co-chaperones which play a part in forming the active ATPase. The C-terminal domain is the carboxyl terminal domain that is having a dimerization motif, which is the second binding region for the drugs and site for the interaction of other co-chaperones. The dimerization

of Hsp 90 monomer via the C-terminus is important for chaperoning function.

1.1.4 Bioinformatics and Computational Biology:

The enhancement of Bioinformatics and Cheminformatics is paving the way for easy drug designing. Computational biology and bioinformatics approach is used to quickly develop new drugs discovery and reduce process cost and changing the process in which the drugs are primarily designed. The rational drug design facilitates and speeds up the drug designing processes that involves various method of identifying novel compounds. One of the important tools for quick and efficient drug discovery is docking the drug molecule with the ligand or target. The drugs bind to the site of attachment and are responsible for the therapeutics effect on the target.

Docking is a method by which two molecules (ligand and receptor) binds to each other in 3D space. There are various tools, software and servers meant for exclusively for the process of docking calculations. There are different databases available online which stores macromolecular 3D structure and ligand structure, which are extracted from NMR and are the co-ordinates used for docking and simulations. Thus computational biology or In Silico approach is developing day by day with new technologies coming every day. It is a promising field that helps to invent drugs to fight various medical conditions.

1.2 OBJECTIVE:

- To search for various Hsp 90 inhibitors from the database.
- In sillico analysis of various Hsp 90 inhibitors to identify the best inhibitor among them all for the therapy of acute myeloid leukemia with flt3-itd (internal tandem duplication).

CHAPTER 2

LITERATURE REVIEW

2.1 Structure and Function of FLT3:

FLT3, belongs to the class of receptor tyrosine kinase family of proteins. It is a membrane bound receptor with an intrinsic TK domain, which is composed of immunoglobulin-like extracellular ligand-binding domain, a trans membrane domain, a juxtamembrane dimerization domain and a very highly conserved intercellular kinase domain with interruptions from kinase inerts.it is expressed on myeloid and lymphoid progenitors (Rosnet et al., 1996).It has been found in lymphohematophoetic organs (Lymann et el,1998).Under normal conditions it exists as monomers,unphosphorylated with an inactive kinase domain. When it interacts with the receptor, a conformational change takes place which results in the unfolding of the receptor and which in turn leads to the exposure of the dimerization domain and it allows interaction between receptor-receptor domain which leads to activation of TK enzyme, leading to phosphorylation of various sites in the intracellular domain. Many other proteins get recruited by the activated receptor in the cytoplasm (SHC, GRB2,GAB2,SHIP,CBL,CBLB,etc.(Dosil et al., 1993),which forms complex protein-protein interaction. These proteins get activated and causes phosphorylation reaction leading to activation of secondary mediators like STAT, MAP kinase,AKT/PI3 kinase signal transduction pathways. On activation these mediators gets chaperoned by Hsp 90.th message trans located in the nuclear interphase triggers a series of events of cell cycle regulation(differentiation ,proliferation,apoptosis,survival).This makes Hsp 90 as an important target to therapeutic approaches for preventing AML with FLT3 internal tandem duplication.

2.2 FLT3 Function in Normal and Malignant Hematopoiesis:

The activation of FLT3 regulates a number of cellular process, and by these processes, FLT3 activation performs a crucial task of governing normal hematopoiesis and cellular growth.(Ray et al., 1996) For FLT3 to function optimal ,it requires the coordination with other growth factors like IL3 and SCF (Rusten et al.1996).The combination of FLT and other growth factors have a role in promoting the hematopoietic progenitor cells (primitive) (Namikawa et al., 1996). FL stimulation appears to mediate differentiation of the early progenitors, where exposure of the hematopoietic progenitors to FL, leads to monocytic differentiation, without significant proliferation. Expression of FLT3 has been evaluated in hematologic malignancies. The majority of B-cell ALL and AML blasts (> 90%) express FLT3 at various leves. Although less frequently and with more variable expression levels, the receptors FLT3 are also expressed in other

malignancies(hematopoietic), including chronic myeloid leukemia (CML),myelodysplasia (MDS), T-cell ALL, and chronic lymphocyctic leukemia (CLL) (rosnet et al., 1996).

2.3 **Genomic Alteration of FLT3 in AML:**

Two distinct mutations were identified in the studies evaluating the genomic alterations in FLT3 which alters its function. INTERNAL TANDEM DUPLICATION (ITD) of the juxtamembrane domain in FLT3 were identified in large number of patients suffering from acute myeloid leukemia (AML) (Nakao et al., 1996). Studies also identified missense mutations in the activation loop of the TK (tyrosine kinase) domain of the FLT3 which is termed as FLT3-ALM (Yamamoto et al., 2001).

The mutation of FLT3 results from the segmental duplication of a fragment inside the juxtamembrane coding region (encode by exon 14 and 15) of the FLT3 protein, which is the most common type of mutations found in hematologic malignancies (like CML, MDS, AML). The prevalence of FLT3 is highly age dependent ranging from rare in case of adults and increase when a person ages. There also exists a considerable variability in size, and region of ITD involvement (Stirewalt et al., 2006). FLT3/ITDs have shown to promote ligand-independent receptor dimerization, leading to autonomous phosphoyrylation and constitutive activation of the receptor, culminating in cytokine independent cellular proliferation. It also promotes proliferation by activating multiple signaling pathways that include STAT, RAS/MAPK and the AKT/PI3 kinase pathways (Mizuki et al., 2009). The identification of FLT3 mutations in AML has provided us with novel approaches to manage this disease.

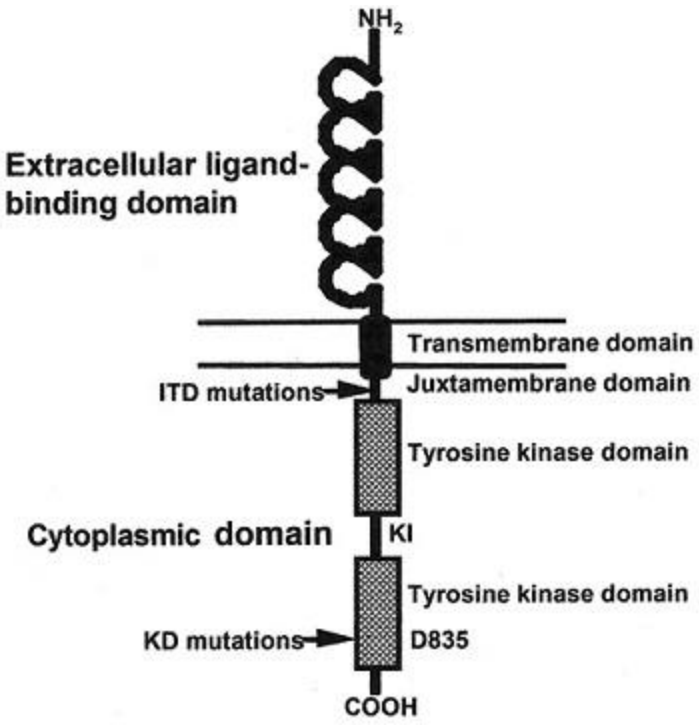


FIGURE 1 : ITD mutation in FLT3 protein (Adapted from reference: John et al.,2006).

2.4 Heat shock proteins:

Under environmental stress, the cells either stop its cycle, or slow down its native functions, such as transportation, RNA, DNA and protein synthesis. A set of proteins, called stress proteins, which are mainly expressed under these adverse conditions of stress response in with increase in the temperature of the environment, called heat shock proteins (Hsp).They are also called molecular chaperones.

Majority of heat shock proteins are categorized as molecular chaperones

Hartl et al., 1996 defined chaperone as "proteins that bind to and stabilize an otherwise unstable form of other protein and, by exact binding and release, enable its correct fate *in vivo*: be it folding, oligomer assembly, transportation to a certain subcellular compartment, or removal by degradation".Multhoff et al., 1996 discovered the expression of heat shock proteins on cancer cell surface.

2.5 Heat Shock Protein 90KDa (Hsp 90):

Hsp 90 is a cytosolic protein, it is present nearly about 1-2% in the cytosol. Its concentration depends on cell type. (Nollen et al., 2002 and Ghaemmaghami et al., 2003). It is over expressed

in cancer cells. (Whitesell et al., 2005 and Pick et al., 2007)

2.6 The structure of Hsp 90:

The eukaryotic structure of Hsp 90 having 40% similarity with its prokaryotic form (Bardwell et al., 1987) and is a dimeric phosphoprotein (Spence et al., 1989).Hsp 90 contains two chaperone-sites, one on its N-terminal domain, and other one on the C-terminal domain. There are also other binding sites. Hsp 90 forms dimers. ATP binds to its N-terminal domain, and a conformational changes occurs. (Minami et al., 1994).

Hsp 90 contains an ATP binding pocket in Its N-terminal domain, where Geldanamycin binds. (Stebbins et al., 1997 and Obermann et al., 1998) and yeast Hsp82 possess an adenine nucleotide binding site (Prodromou et al., 1997, Prodromou et al., 2000). Comparing the structures of N-terminal domain of human Hsp 90 without ligand and with ATP / AMPPCP was given by Li et al., 2012. Human Hsp 90 alpha consists of approximately 732 amino acids, three domains (N-terminal-terminal, middle and C-terminal) and a charge linker. (Huai et al., 2005, Harris et al., 2004 and Dutta et al., 2000).Co-chaperones and client proteins association regulate the ATPase activity of Hsp 90 (Whitesell L. et al., 2005).

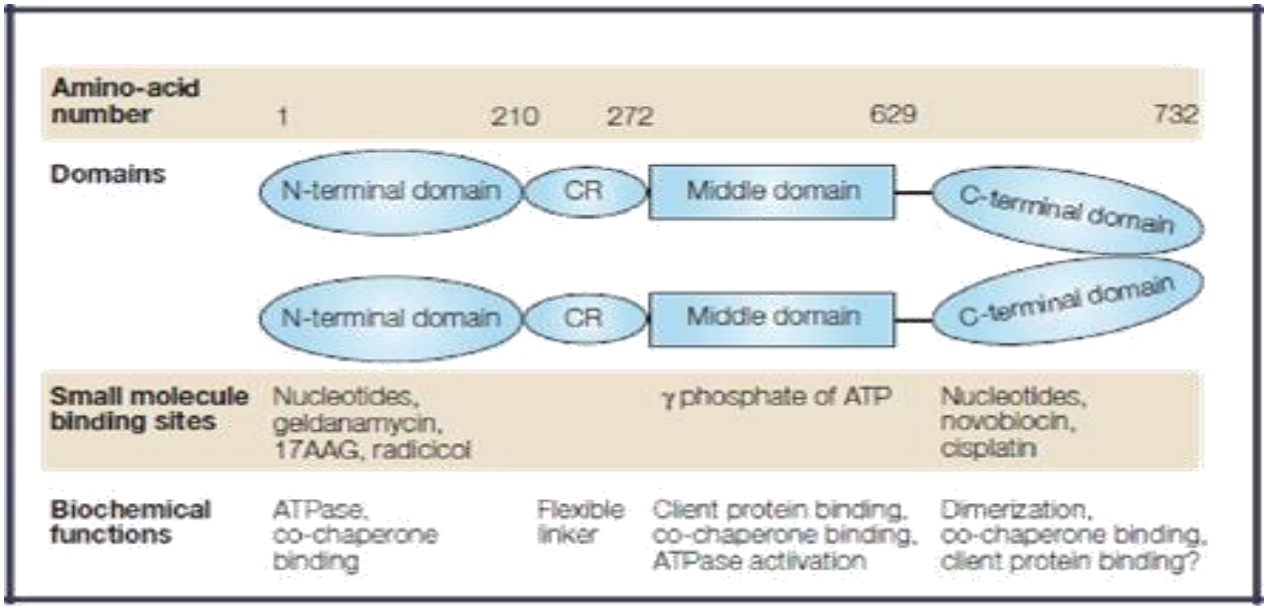


Figure 2: Structure of Hsp 90 (Adapted from Ref: *Whitesell* et al., *2005***).**

2.7 The Functions of Hsp 90 in various biological processes.

Hsp 90 can bind to the target proteins and is essential for their folding, maturation and maintenance. (Hartl et al., 1996, Buchner et al., 1999, and Csermely *et al.,* 1998). Hsp 90 bind to various peptides either in vivo or in vitro. (Menoret et el, 1999) Hsp 90 is involved in signaling processes, and thus poses threat to cellular function. (Blum et al., 2000). Extracellular Hsp 90 interacted with the receptor CD91 (Basu et al., 2001 and Cheng et al., 2008). Role in disassembly of transcriptional complexes: Hsp 90 and p23 are recruited to chromatin-bound glucocorticoid receptor; promoter-bound p23. Hsp 90 inhibits GR, TR, NFκB, an AP-1 (Freeman et al., 2002).

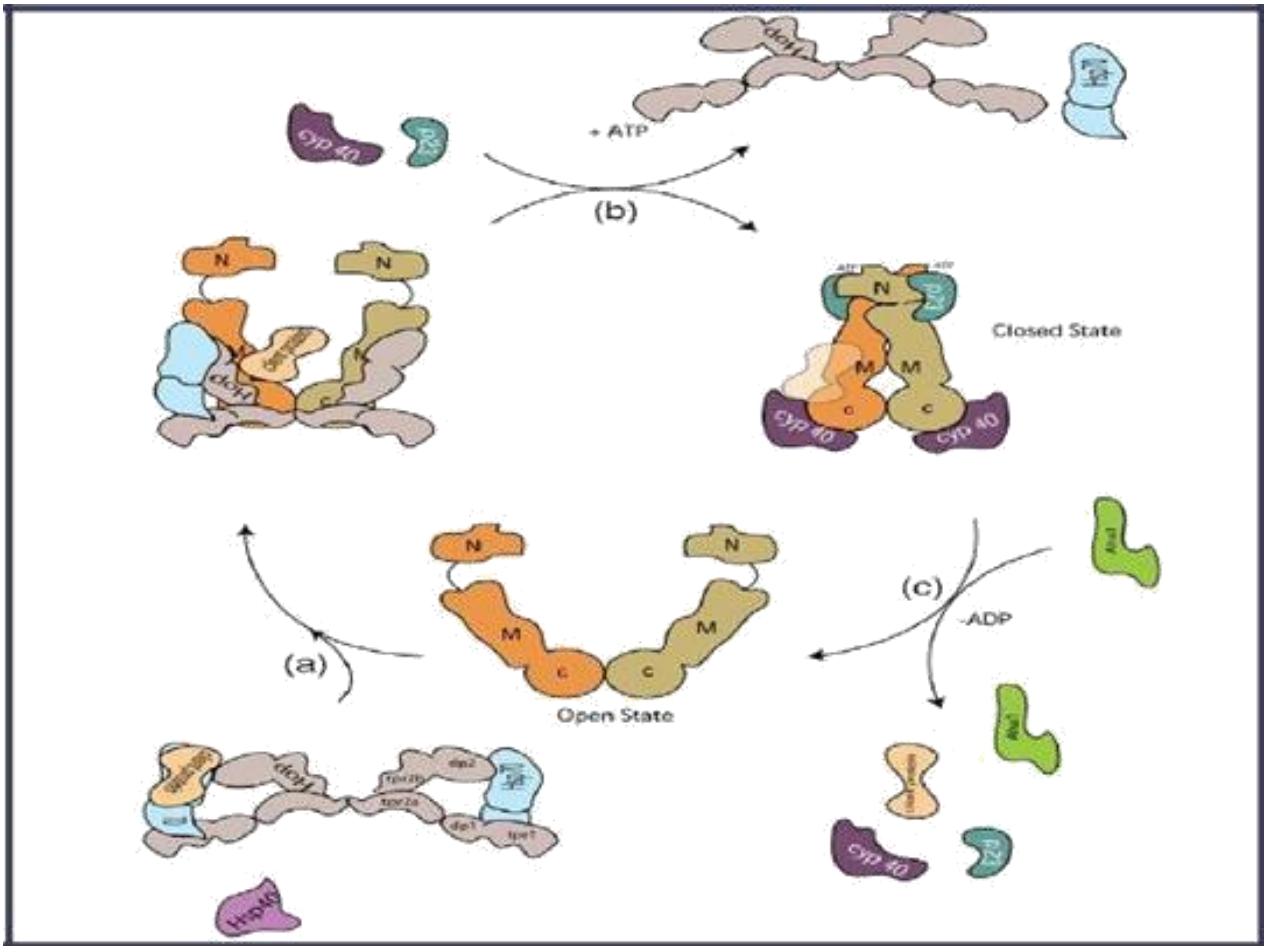


Figure 3: The Model of the conformational cycle of Hsp 90 (Adapted from Ref: *Grossmann* et al., *2008).*

2.8 Hsp 90 Inhibition:

There are various natural and synthetic molecules that have been declared as promising in cancer therapy via disrupting the complex of ATP-HSP 90-client proteins by targeting Hsp 90 N-terminal ATP binding pocket. Geldanamycin was the first inhibitor that led the way for other inhibitors to represent Hsp 90 a therapeutic target for cancer therapy. (Schulte et al., 1997)

Geldanamycin A and Herbimycin A induce degradation of Raf, receptor tyrosine kinases (Schulte et al., 1997), CFTR (Loo et al., 1998) through proteasomes degradation, and transfected nNOS (Bender et al., 1999). Cancer cells with mutant p53 are more sensitive to Hsp 90 inhibitors than cells with wild type p53. Coumarin antibiotics such as Novobiocin bind Hsp 90 and reduce levels of Hsp 90 clients (Marcu et al., 2000). Geldanamycin A derivative WX514 .17AAG ,17DMAG expresses much improve results.(Clarke et al 2000 and Xu et al., 2001) Geldanamycin stimulate association of CHIP with client (erbB2) and facilitates degradation (Xu et al., 2002) and targets tumor cells because of a 100-fold higher affinity of their Hsp 90 complexes (Kamal et al., 2003). A review on Hsp 90 inhibition to elucidate a new strategy for protein kinases has been done. (Sreedhar et al., 2004).

A new class of Hsp 90 inhibitors by structure based drug designing has been discovered. Other novel Hsp 90 inhibitors discovered the natural triterpenoids celastrol and gedunin, by chemical genomics (Hieronymus et al., 2006). Celastrol interrupts Hsp 90 interaction with Cdc37 and function without hindering ATP binding (Zhang et al., 2008 and Zhang et al., 2009).The involvement of computational biology and the docking studies on anticancer drugs,

has been much helped.-A review on all Hsp 90 inhibitors up to date given by HUIFANG HAO 2010, Detailed thermodynamic analysis of drug binding to human and yeast Hsp 90 (Zubrienė et al., 2010). GA/17AAG target VDAC resulting in membrane depolarization of mitochondria and increased intracellular Ca2+ (Xie et al., 2011). 3D structure elucidation and macromolecular interactions on Hsp 90 help in establish drug designing (Madej et al., 2011).

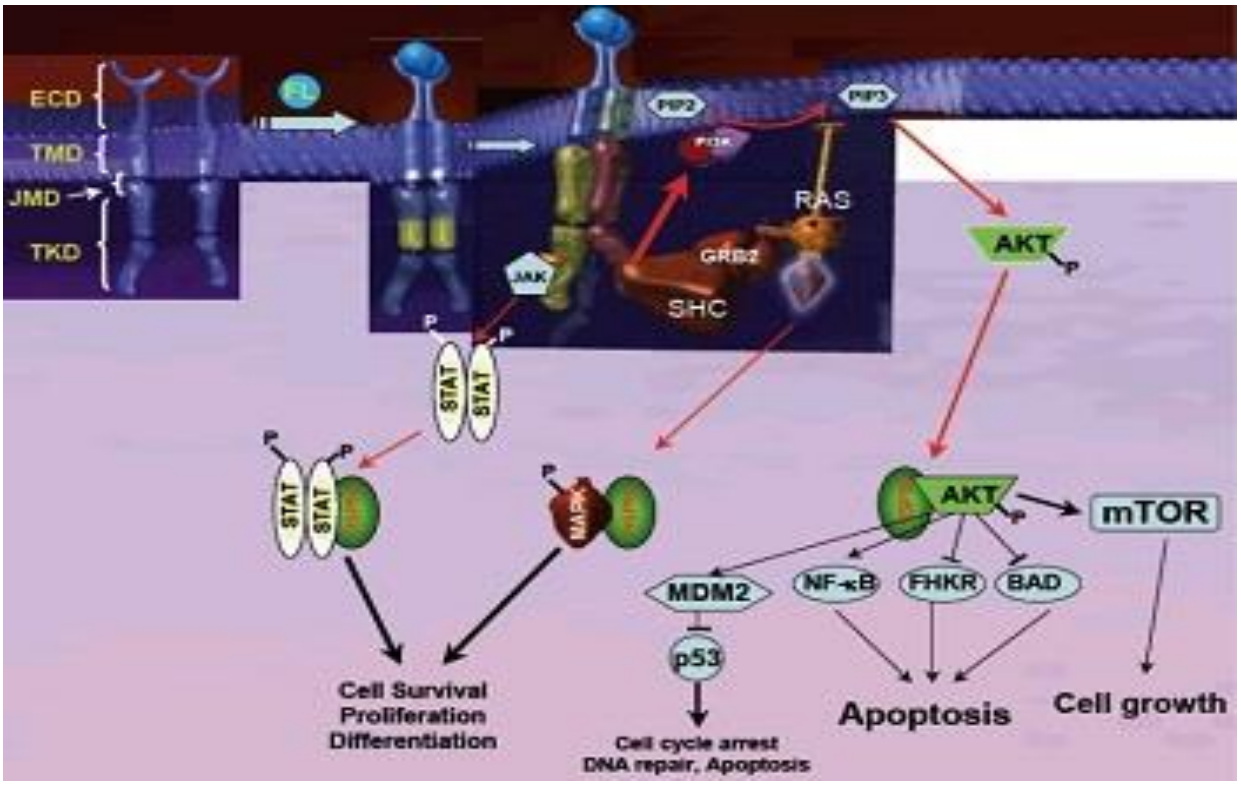


Figure 4:FLT3 signal transduction pathway**(**Adapted from Ref: Soheil et al., *2009).*

CHAPTER 3

TOOLS AND METHODS

3. Tools and Methods:

3.1.1 Tools and Bioinformatics softwares used:

- Swiss-PdbViewer
- Argus Lab 4.0.1
- Auto Dock 4.0
- Auto DockVina 1.0
- Chimera 1.6.1
- MGL Tools
- Open Babel
- Pymol

3.1.2 Online server's used:

- http://www.rcsb.org/
- https://pubchem.ncbi.nlm.nih.gov/
- http://www.swissdock.ch/
- http://projects.biotec.tu-dresden.de/metapocket/
- http://uniprot.org
- http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg

3.1.3 Files Required:

- PDB file of targeted protein [Hsp 90]
- SDF files of Hsp 90 Inhibitors
- PDB files of Hsp 90 Inhibitors using Open Babel
- PDBQT files of both target protein and Its Inhibitors

3.2 PROTOCOL FOLLOWED:

In this study we have selected hsp 90**(PDB ID:1YET)** as the target because it is up regulated in patients with AML with FLT3-ITD mutations. So the inhibition of hsp 90 will help in apoptosis of cancer cells.

3.2.1 STEPS IN INHIBITION OF HSP 90:

1. Retrieval of the 3D structure Hsp 90 dimer protein from rcsb.org PDB (1YET).

2. Collection of SDF files of Hsp 90 Inhibitors molecule from PubChem database.

3. Energy minimization of inhibitor by using ProDrg sever

4. Conversion of SDF files into PDB files by using Open Babel software

5. PDB files of Both Protein [Hsp 90] and Ligand Hsp 90 inhibitors are converted into PDBQT file Using Auto Dock 4.0

6. Docking of all Hsp 90 inhibitor with Hsp 90 dimer molecule is done using AutoDockVina

7. Analysis of the binding energy results obtained from the molecular docking

3.3 Methodology:

3.3.1 Retrieval of amino acid sequences of Hsp 90 protein from NCBI:

NCBI stands for National Centre for Biotechnological Information. It is established as a division of National Library of Medicines at National Institutes of Health. The NCBI responsible for creating automated systems of knowledge about molecular biology, biochemistry, and genetics and thus providing the use of the databases and software tools by the researchers and medical professionals; collect biotechnology information globally; and execution research on advanced methods of in sillico information processing for examining the structure and function of biological molecules which are of importance. The URL for this database is http://www.ncbi.nlm.nih.gov.

3.3.2 Retrieval of 3D structure of Hsp 90 Protein modelled by PHYRE server:

PHYRE is an automatic fold recognition server which is used for calculation of the structure and function of the protein sequence that is submitted to the server. It is for academics use only. It based on the principle of Homology Modelling and relies on Hidden Markov Models.

1. The Fasta format of amino acid sequences was pasted and Phyre 2 search was clicked.
2. After 5 hours the results were sent to the users given email address.

Figure 5: Submission of amino acids in PHYRE server

3.3.3 Retrieval of 3-D protein structure ATP from PDB (Protein Data bank):

The PDB (Protein Data Bank) is the universal store of Structural data of Biological macromolecules, founded in Brookhaven National Laboratories (BNL) in 1971 which provides Structural information of the macromolecules assessed by X-ray crystallographic, NMR Methods. Understanding the shape tells us about the functions. As biological macromolecule like protein is having a structure to function relationship. Hence an accurate knowledge of structure is needed to know the various functions. We can download the structure in pdb file format or fasta format from the server.

i. The URL www.pdb.org was browsed.

ii. In the search option Individual protein name was typed.

iii. After selecting the required PDB ID, the structure was downloaded and saved in .pdb format.

3.3.4 Energy minimization of all 3D structure of proteins by Chimera 1.7:

UCSF CHIMERA 1.7 is an extensible programming tool for visualization and analysis of Molecular structure and related data including density maps, supramolecular associations, Sequence alignments, docking results, routes and conformational ensembles. One of the best Features this tool provide is the structural editing job, which minimizes the energy of molecules providing them high stability.

- The Chimera window was opened.
- Go to the option file, the 3D structure of protein was retrieved.
- The total residues were selected.
- From the tool option, by the structure editing option, minimized structure option was Clicked.
- The minimized structure was saved in .pdb format.

3.3.5 Geometry Optimization of all 3D structure of proteins by ArgusLab 4:

Argus Lab software is used for the geometry optimization of the Protein molecule which we need to dock. This is done so that when we perform docking it gives a perfect result without any errors. It optimize the geometry for better orientation.

STEPS

- ArgusLab 4.0 window was opened.
- From the option file, the 3D structure of protein was retrieved.
- Then go to geometry optimization tools.
- From the tool option, by the structure editing option, Optimize geometry option was clicked.
- The Optimized structure was saved in .pdb format.

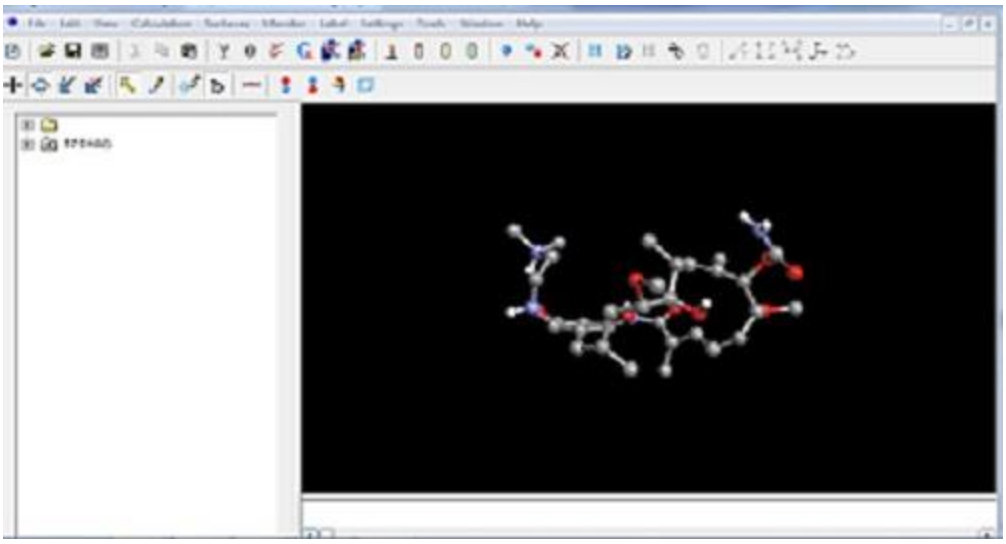


Figure 6: Geometry Optimization of Hsp 90's dimer molecule in ArgusLab 4.0.

3.3.6 Collection of small ligand molecules from Pubchem:

Pubchem is a database of chemical structures of smaller organic molecules and contain information related to their biological activity, origin and its related literatures. It is updated by NCBI. Millions of compound structures and relevant data can be freely downloaded from PUBCHEM.

Pubchem page was retrieved by browsing Pubchem.ncbi.nlm.nih.gov.

1. In search bar individual inhibitors name was typed and entered.
2. All the available ligands of therapeutic target database are retrieved in .sdf file format.
3. The ligands retrieved were taxol,venblastin,radicicol,clorobocin,herbimycin a,geldanamycin,novobiocin,macbecin 1,gantespib,coumermycin A,17-DMAG,.

3.3.7 Conversion of .sdf file format to .pdb by Open Babel GUI:

Open Babel 2.3.1 is a chemical toolkit designed to interpret the various language of chemical Data It searches, analyses and converts chemical data. It covert chemical data from one file format to another file format.

 Open Babel 2.3.1 window was opened.

 The input format was selected as .sd and the output format as .pdb.

 From the input option .sd file was browsed.

 Click on the convert option to convert .sd file format to .pdb file format.

 Then the selected files were generated in the .pdb file format and save it for future use.

3.3.8 Conversion of .pdb to .pdbqt of Molecules Using Auto Dock 4.0:

Auto Dock 4.0 is a molecular graphics program hosted and devenloped by The Scripps Research Institute for estimating docking calculations and displaying docking results of protein and ligand molecules.

3.3.8.1 Covert .pdb of Hsp 90 molecule to .pdbqt:

Step by step commands:-

FILE > READ MOLECULE > EDIT > HYDROGEN > ADD POLAR CHARGE > EDIT > CHARGES> ADD KOLLMAN CHARGES > EDIT >ATOMS > ASSIGN AD4 TYPE> FILE > SAVE >WRITE.PDBQT.

3.3.8.2 Covert .pdb of Inhibitor molecule to .pdbqt:

step by step command

LIGAND>INPUT MOLECULES> READ MOLECULE>LIGAND >TORSION TREE>LIGAND>CHOOSE ROOT>LIGAND >DETECT ROOT>LIGAND>OUTPUT> SAVE AS .PDBQT.

3.3.9 Molecular Docking Using Auto DockVina:

It is a molecular docking tool.

Steps

- Open the Command prompt in the system.
- Give the location of the .pdbqt files of both Protein and inhibitor in cmd.
- Then give the location of program vina in the program file for processing
- Create a conf.txt file giving the information of configuration for the Docking.
- Give the command in the cmd for docking procedure to initiate.
- Result is displayed in the log file generated by the software.

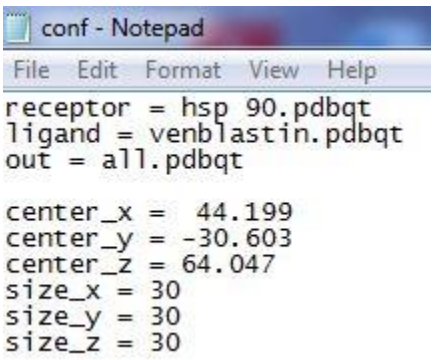
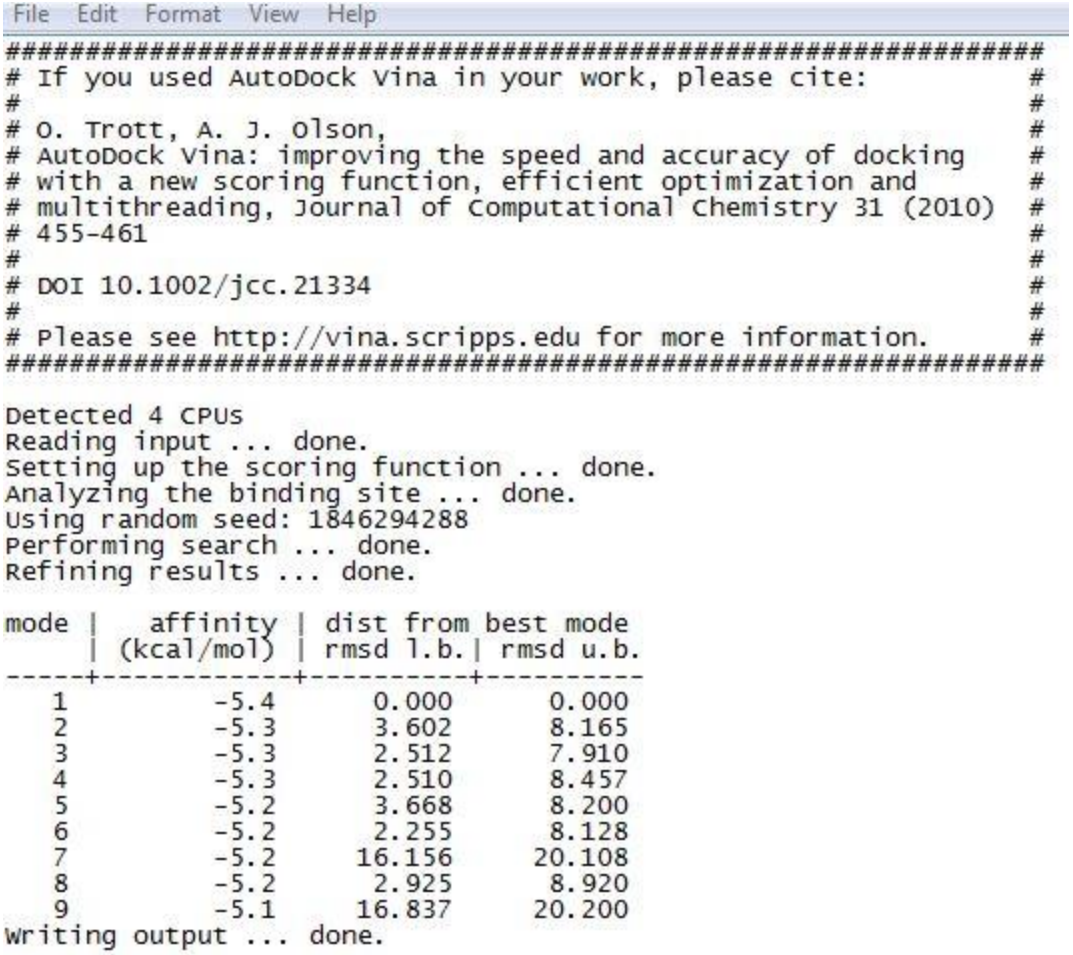


Figure 7: Config file for Hsp 90 protein's locations for docking..



```
File  Edit  Format  View  Help
#################################################################
# If you used AutoDock Vina in your work, please cite:          #
#                                                               #
# O. Trott, A. J. Olson,                                        #
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################

Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1846294288
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -5.4      0.000      0.000
   2         -5.3      3.602      8.165
   3         -5.3      2.512      7.910
   4         -5.3      2.510      8.457
   5         -5.2      3.668      8.200
   6         -5.2      2.255      8.128
   7         -5.2     16.156     20.108
   8         -5.2      2.925      8.920
   9         -5.1     16.837     20.200
Writing output ... done.
```

Figure 8: Result log file for Hsp 90's protein and inhibitor for docking.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Results:

4.1.1 Docking results of Hsp90 –ATP molecule:

Hsp90 was docked with ATP molecule at various binding sites,the docking results are summarized in Table 1 .The findings of the results are solely based on the docking energy value and the interaction at the binding sites. The more negative the value, the more stable the complex is and more binding affinity. According to the energy funnel theory less energy depicts highly stable conformation. Hence more energy would be needed to break the complex that means high dissociation energy.

TABLE 1. Docking of Hsp 90 with ATP.

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	ATP	51	-5.5
		93	-5.5
		112	-5.6
		138	-5.4
		400	**-5.9**

4.1.2 Inhibition of Hsp 90:

All the ligands from various databases were docked with Hsp90 and the results tabulated below.

TABLE 2. Docking of Hsp 90 with 17-DMAG

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	17-DMAG	51	-5.2
		93	-5.4
		112	-5.0
		138	-5.3
		400	**-5.4**

TABLE 3. Docking of Hsp 90 with 17-GMB-APA-GA.

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	17-GMB-APA-GA	51	-6.0
		93	-5.5
		112	-5.3
		138	-5.7
		400	**-6.7**

TABLE 4. Docking of Hsp 90 with A4.

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	A4	51	-5.1
		93	-5.1
		112	-5.0
		138	-5.2
		400	**-5.5**

TABLE 5. Docking of Hsp 90 with AUY-922.

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	AUY-922	51	**-5.6**
		93	-5.2
		112	-5.4
		138	-5.4
		400	-5.3

TABLE 6. Docking of Hsp 90 with CLOROBIOCIN.

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	CLOROBIOCIN	51	-6.6
		93	-6.6
		112	-6.3
		138	-6.8
		400	**-7.2**

TABLE 7. Docking of Hsp 90 with COUMERMYCIN A1.

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	COUMERMYCIN A1	51	-7.0
		93	-7.4
		112	-7.8
		138	**-8.1**
		400	-7.9

TABLE 8. Docking of Hsp 90 with GANTESPIB.

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	GANTESPIB	51	-5.7
		93	-5.7
		112	-5.4
		138	-5.8
		400	-5.8

TABLE 9. Docking of Hsp 90 with GELDANAMYCIN.

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	GELDANAMYCIN	51	-5.3
		93	-5.5
		112	-5.5
		138	-5.5
		400	**-6.9**

TABLE 10. Docking of Hsp 90 with HERBIMYCIN A.

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	HERBIMYCIN A	51	-5.2
		93	-4.9
		112	-4.9
		138	-4.7
		400	**-5.6**

TABLE 11. Docking of Hsp 90 with MACBECIN I.

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	MACEBIN I	51	-5.4
		93	-5.0
		112	-4.8
		138	-4.7
		400	**-5.8**

TABLE 12. Docking of Hsp 90 with NOVOBIOCIN.

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	NOVOBIOCIN	51	-5.9
		93	-5.7
		112	-6.1
		138	-6.3
		400	**-6.9**

TABLE 13. Docking of Hsp 90 with RADICICOL.

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	RADICICOL	51	**-6.1**
		93	-5.8
		112	-5.8
		138	-5.3
		400	-5.9

TABLE 14. Docking of Hsp 90 with TANESPIMYCIN

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	TANESPIMYCIN	51	**-6.0**
		93	-5.2
		112	-5.3
		138	-4.9
		400	-5.8

TABLE 15. Docking of Hsp 90 with TAXOL.

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	TAXOL	51	**-6.2**
		93	-6.1
		112	-5.5
		138	-6.1
		400	-5.9

TABLE 16. Docking of Hsp 90 with VENBLASTIN

PROTEIN	LIGAND	ACTIVE SITE	BINDING AFFINITY(Kcal/mol)
HSP 90	VENBLASTIN	51	**-6.2**
		93	-6.1
		112	-5.5
		138	-6.1
		400	-5.9

4.2 DISCUSSIONS:

The analysis of the binding energy data showed that the inhibitors gave best results at binding site 400 in general. Coumermycin A1 showed the best result with binding energy of -8.1 kcal/mol at the binding site 138 of the Hsp 90.

Clorobiocin was yet another compound that showed promising results with energy value of -7.2 kcal/mol at binding site 400.

Novobiocin was another compound which showed good results and is one of the most widely used inhibitor in clinical trials.

CHAPTER 5

CONCLUSION AND FUTURE PERSPECTIVE

5.1 CONCLUSION:

Interest in Hsp 90 inhibitors for the treatment of cancer and neurodegenerative diseases has grown exponentially since the identification of the compound Geldanamycin as the very first Hsp 90 inhibitor in the year1994. In the last 3 decades, several classes and types of Hsp 90 inhibitors has been discovered, with each having unique mechanisms of inhibition and each showing different effect on the biological system. Many of these inhibitors have been clinically tested for different types of cancers, but, the results of these clinical trials have been disappointing to some extent.the emphasizes on the need to understand the full biology of the target moleculesand how different types and classes of inhibitor affect the chaperoning activity.

Another approach can be directly inhibiting the FLT3 protein which will down regulate it. There are many compounds available which can directly bind to flt3 and cause its inhibition eg. Lestaurtinib, Midostaurin etc.

5.2 Future perspective:

The above findings were established with the help of promising, highly developed and reliable tools of computational biology. In this modern era of Insilco, every work is first tested by Virtual screening or Insilico designing, then only it goes for invitro and invivo analysis. The protocol follows like Insilico designing, Insitu designing, Invitro analysis and final invivo analysis.

The crucial biological functions as performed by Hsps90 and the dependency of carcinogenic cells on the known functions of Hsp 90 make itself as an important target for anti-cancer therapy. The trademarks of cancer, an up-regulation of growth signals and apoptotic interruption are the most important ones. As maximum growth signals rely on Hsp 90 for their functionality and stability, Hsp 90 become is considered an ideal molecule to interfere in complicated mechanism of pathways for oncogenes. Therefore, drugs targets for Hsp 90 are more of more advantage than focusing on the oncogenic pathway inhibitors.

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